

What is claimed is:

1. A mixed sequence oligonucleotide comprising at least 12 nucleotides in length and having a 3' end and a 5' end and divided into a first portion and a further portion,
5 said first portion being capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide,
said further portion being incapable of supporting said cleavage by said RNase H1;
10 wherein said first portion comprises at least 6 nucleotides and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is 8 to 12 nucleotides from the 3' end of said oligonucleotide.
2. The oligonucleotide of claim 1 comprising at
15 least one CA nucleotide sequence within said first portion.
3. The oligonucleotide of claim 1 comprising from about 12 to about 50 nucleotides.
4. The oligonucleotide of claim 1 comprising from about 12 to about 25 nucleotides.
- 20 5. The oligonucleotide of claim 1 wherein each of said nucleotides of said first portion have B-form conformational geometry and are joined together in a continuous sequence.
6. The oligonucleotide of claim 1 wherein each of
25 said nucleotides of said first portion is, independently, a 2'-deoxyribonucleotide, a 2'-SCH₃ ribonucleotide, a 2'-NH₂ ribonucleotide, a 2'-NH(C₁-C₂ alkyl) ribonucleotide, a 2'-N(C₁-C₂ alkyl)₂ ribonucleotide, a 2'-CF₃ ribonucleotide, a

2'=CH₂ ribonucleotide, a 2'=CHF ribonucleotide, a 2'=CF₂ ribonucleotide, a 2'-CH₃ ribonucleotide, a 2'-C₂H₅ ribonucleotide, a 2'-CH=CH₂ ribonucleotide or a 2'-C≡CH ribonucleotide.

5 7. The oligonucleotide of claim 1 wherein each of said nucleotides of said first portion is a 2'-deoxyribonucleotide.

8. The oligonucleotide of claim 1 wherein each of said nucleotide of said first portion is, independently, a
10 2'-CN arabinonucleotide, a 2'-F arabinonucleotide, a 2'-Cl arabinonucleotide, a 2'-Br arabinonucleotide, a 2'-N₃ arabinonucleotide, a 2'-OH arabinonucleotide, a 2'-O-CH₃ arabinonucleotide or a 2'-dehydro-2'-CH₃ arabinonucleotide.

9. The oligonucleotide of claim 1 wherein each of
15 said nucleotides of said first portion is, independently, a 2'-F arabinonucleotide, a 2'-OH arabinonucleotide or a 2'-O-CH₃ arabinonucleotide.

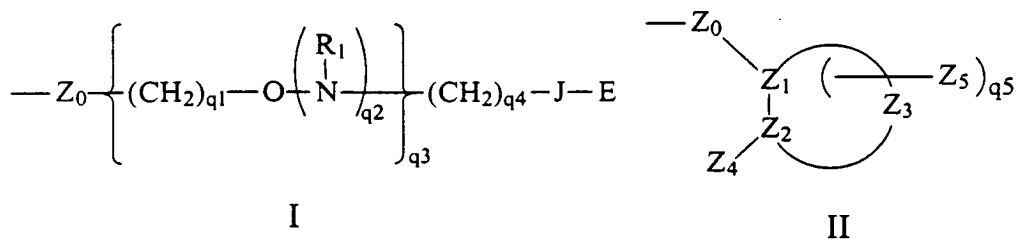
10. The oligonucleotide of claim 1 wherein each of
20 said nucleotides of said first portion is, independently, a 2'-F arabinonucleotide or a 2'-OH arabinonucleotide.

11. The oligonucleotide of claim 1 wherein said nucleotides of said first portion are joined together in said continuous sequence by phosphate, phosphorothioate, phosphorodithioate or boranophosphate linkages.

25 12. The oligonucleotide of claim 1 wherein said further portion includes a plurality of nucleotides, at least some of said nucleotides comprise a 2' substituent group wherein each substituent group is, independently,

hydroxyl, C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₂-C₂₀ alkynyl, halogen, amino, thiol, keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, O-alkyl, O-alkenyl, O-alkynyl, S-alkyl, S-alkenyl, S-alkynyl, NH-alkyl, NH-alkenyl, NH-alkynyl, N-dialkyl, O-aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-aralkyl, N-phthalimido, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, aryl, heterocycle, carbocycle, intercalator, reporter molecule, conjugate, polyamine, polyamide, polyalkylene glycol, or polyether;

or each substituent group has one of formula I or II:

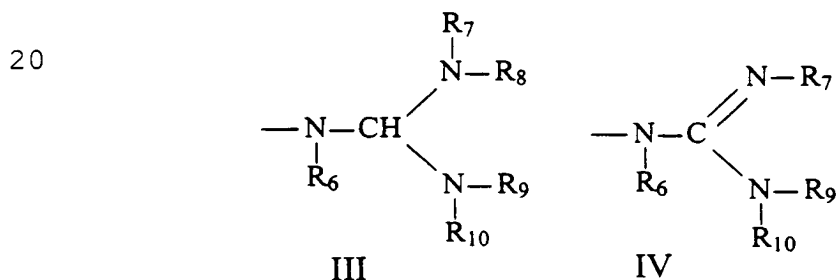


wherein:

Z₀ is O, S or NH;

J is a single bond, O or C(=O);

E is C₁-C₁₀ alkyl, N(R₁)(R₂), N(R₁)(R₅), N=C(R₁)(R₂), N=C(R₁)(R₅) or has one of formula III or IV;



each R₆, R₇, R₈, R₉ and R₁₀ is, independently, hydrogen, C(O)R₁₁, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, alkylsulfonyl, arylsulfonyl, a

chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

5 or optionally, R₇ and R₈, together form a phthalimido moiety with the nitrogen atom to which they are attached;

or optionally, R₉ and R₁₀, together form a phthalimido moiety with the nitrogen atom to which they are attached;

each R₁₁ is, independently, substituted or

10 unsubstituted C₁-C₁₀ alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

R₅ is T-L,

15 T is a bond or a linking moiety;

L is a chemical functional group, a conjugate group or a solid support material;

each R₁ and R₂ is, independently, H, a nitrogen protecting group, substituted or unsubstituted C₁-C₁₀ alkyl, 20 substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, wherein said substitution is OR₃, SR₃, NH₃⁺, N(R₃)(R₄), guanidino or acyl where said acyl is an acid amide or an ester;

or R₁ and R₂, together, are a nitrogen protecting group 25 or are joined in a ring structure that optionally includes an additional heteroatom selected from N and O;

or R₁, T and L, together, are a chemical functional group;

each R₃ and R₄ is, independently, H, C₁-C₁₀ alkyl, a 30 nitrogen protecting group, or R₃ and R₄, together, are a nitrogen protecting group;

or R₃ and R₄ are joined in a ring structure that optionally includes an additional heteroatom selected from N and O;

Z_4 is OX, SX, or $N(X)_2$;

each X is, independently, H, C_1-C_8 alkyl, C_1-C_6 haloalkyl, $C(=NH)N(H)R_5$, $C(=O)N(H)R_5$ or $OC(=O)N(H)R_5$;

R_5 is H or C_1-C_8 alkyl;

5 Z_1 , Z_2 and Z_3 comprise a ring system having from about 4 to about 7 carbon atoms or having from about 3 to about 6 carbon atoms and 1 or 2 hetero atoms wherein said hetero atoms are selected from oxygen, nitrogen and sulfur and wherein said ring system is aliphatic, unsaturated
10 aliphatic, aromatic, or saturated or unsaturated heterocyclic;

Z_5 is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about 10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about
15 14 carbon atoms, $N(R_1)(R_2)OR_1$, halo, SR_1 or CN;

each q_1 is, independently, an integer from 1 to 10;

each q_2 is, independently, 0 or 1;

q_3 is 0 or an integer from 1 to 10;

q_4 is an integer from 1 to 10; and

20 q_5 is from 0, 1 or 2;

provided that when q_3 is 0, q_4 is greater than 1.

13. The oligonucleotide of claim 1 wherein each of said nucleotides of said further portion is, independently, a 2'-F ribonucleotide, a 2'-O- $(C_1-C_6$ alkyl) ribonucleotide,
25 or a 2'-O- $(C_1-C_6$ substituted alkyl) ribonucleotide wherein the substitution is C_1-C_6 ether, C_1-C_6 thioether, amino, amino(C_1-C_6 alkyl) or amino(C_1-C_6 alkyl)₂.

14. The oligonucleotide of claim 1 wherein said
30 nucleotides of said further portion are joined together in a continuous sequence by 3'-5' phosphodiester, 2'-5' phosphodiester, phosphorothioate, Sp phosphorothioate, Rp phosphorothioate, phosphorodithioate, 3'-deoxy-3'-amino

phosphoroamidate, 3'-methylenephosphonate, methylene(methylimino), dimethylhydrazino, amide 3, amide 4 or boranophosphate linkages.

15. The oligonucleotide of claim 1 wherein at least
5 two of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 3' to said first portion.

16. The oligonucleotide of claim 1 wherein at least
10 two of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

17. The oligonucleotide of claim 1 wherein at least
two of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 3' to
15 said first portion and at least two of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

18. The oligonucleotide of claim 1 wherein at least
four of said nucleotides of said further portion are joined
20 together in a continuous sequence that is positioned 3' to said first portion.

19. The oligonucleotide of claim 1 wherein at least
four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 5' to
25 said first portion.

20. The oligonucleotide of claim 1 wherein at least
four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 3' to

said first portion and at least four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

21. A mixed sequence oligonucleotide comprising at least 8 nucleotides and having a CA nucleotide sequence wherein at least one of the two nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

22. The oligonucleotide of claim 21 wherein said oligonucleotide is capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide.

23. A mixed sequence chimeric oligonucleotide comprising at least 8 nucleotides and having a CA nucleotide sequence wherein at least one of the two nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

24. The chimeric oligonucleotide of claim 23 wherein said oligonucleotide is capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide.

25. A mixed sequence oligonucleotide comprising 8 to 25 nucleotides and having a CA nucleotide sequence wherein at least one of the nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

26. A mixed sequence chimeric oligonucleotide comprising 8 to 25 nucleotides and having a CA nucleotide sequence wherein at least one of the nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end

of said oligonucleotide.

27. A chimeric oligonucleotide comprising 8 to 25 nucleotides and having a portion capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide wherein said portion supporting said cleavage is at least 6 nucleotides in length and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

10 28. The oligonucleotide of claim 27 wherein said oligonucleotide comprises at least one CA nucleotide sequence within said portion supporting said cleavage.